UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Office of Prevention, Pesticides
and
Toxic Substances

TXR#: 0050705

DATE: May 9, 2002

MEMORANDUM

SUBJECT: **CARBARYL - 6th Report** of the Hazard Identification Assessment Review Committee.

FROM: Virginia A. Dobozy, VMD, MPH
RRB1
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Jeff Dawson, Risk Assessor
RRB1
Health Effects Division (7509C)

PC Code: 056801

On April 25, 2002, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Carbaryl with regard to the Data Evaluation Reports for three dermal toxicity studies and the endpoints for short- and intermediate-term incidental oral exposure and short-, intermediate- and long-term dermal exposure for occupational/residential risk assessments. The conclusions drawn at this meeting are presented in this report.

Committee Members in Attendance

Members present were: Ayaad Assaad, Jonathan Chen, Paula Deschamp, Elizabeth Doyle, Virginia Fornillo, Pamela Hurley, John Liccione, Elizabeth Mendez, David Nixon, Jess Rowland

Member(s) in absentia: William Burnam

Data evaluation prepared by: Virginia A. Dobozy, VMD, MPH

Also in attendance were: Jeff Dawson, Michael Metzger, Whang Phang, William Sette, Anthony Britten (SRRD)

Data Evaluation / Report Presentation

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Virginia A. Dobozy, VMD, MPH
Toxicologist

INTRODUCTION

On April 25, 2002, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Carbaryl with regard to the Data Evaluation Reports (DERs) for three dermal toxicity studies and the endpoints for short- and intermediate-term incidental oral exposure and short-, intermediate- and long-term dermal exposure for occupational/residential risk assessments. The conclusions drawn at this meeting are presented in this report.

The HIARC has met to establish endpoints for risk assessment and determine the FQPA Safety Factor for Carbaryl on five previous occasions. The most recent meeting was on February 19, 2002. At that time, endpoints for dermal exposure were based on oral studies as no dermal toxicity studies were available. In addition, the durations for the short-, intermediate- and long-term dermal exposure, along with short- and intermediate-term incidental oral exposure, were not updated to the current policy (June 4, 2001 Memorandum from HED Division Director) because dermal studies were expected to be submitted by the registrant in the near future. Those studies, one with technical chemical and two with formulations, have now been submitted and reviewed.

RRB1 requested that the HIARC review the following:

1) DERs for dermal studies

The registrant submitted three 4-week dermal toxicity studies, one with the technical chemical and two with formulations (44.82% liquid and 80% powder). None of the studies were conducted according to the 21/28-day dermal toxicity guidelines (OPPTS 870.3200). The following parameters were measured in the technical study: clinical observations, body weight, body weight gain, food consumption, RBC and brain cholinesterase (ChE) and signs of dermal irritation. The same parameters, with the exception of brain ChE, were measured in the formulation studies.

The studies were difficult to interpret and classify for several reasons. Determination of the NOAEL/LOAEL was difficult because: 1) there was no dose response at most of the time periods in the technical study; 2) statistically significant, but minimal, effects were observed; and 3) there was no correlation of brain and RBC ChE inhibition in the technical study (i.e., there was no RBC ChE inhibition during the latter part of the study but brain ChE was decreased in the mid- and high-dose groups at termination). Study classification was difficult because many parameters were not measured. RRB1 concluded that the technical study, while non-guideline, was acceptable and could be used for risk assessment. However, the two formulation studies were classified as unacceptable due to inconsistency of results and lack of brain and plasma ChE measurements.

RRB1 requested that the HIARC review the studies and provide an opinion about the NOAEL/LOAELs, endpoints and study classification. **The HIARC agreed with the conclusions of the DERs and the classification of the studies. The dermal toxicity study with the technical chemical (MRID 45630601) was classified as acceptable. The systemic LOAEL was conservatively established at 50 mg/kg/day based on statistically significant decreases in RBC cholinesterase in males and females and brain cholinesterase in males. The systemic NOAEL was 20 mg/kg/day. There was no**

evidence of dermal irritation at any of the doses tested. Both of the dermal toxicity studies with formulations (MRIDs 45630602 and 4563603) were classified as unacceptable because the most sensitive endpoint (brain cholinesterase inhibition) was not measured.

2) Studies and endpoints proposed for the short-, intermediate- and long-term dermal exposure scenarios based on the recently submitted dermal toxicity studies.

3) Studies and endpoints proposed for short- and intermediate-term incidental oral exposure endpoints based on the updated durations of exposure.

I. FQPA HAZARD CONSIDERATIONS

(Included from February 19, 2002 meeting for completeness)

1. Adequacy of the Toxicity Data Base

The HIARC concluded that the toxicology database for Carbaryl is complete for FQPA assessment. The following acceptable studies are available:

-- Acute delayed neurotoxicity study in hen
-- Acute and subchronic neurotoxicity studies in rats
-- Developmental toxicity studies in rats and rabbits
--Multi-generation reproduction study in rats
-- Developmental neurotoxicity study in rats

2. Evidence of Neurotoxicity

The HIARC concluded that there is a concern for neurotoxicity resulting from exposure to Carbaryl. Evidence of neurotoxicity was observed in the following neurotoxicity studies, as well as in the chronic dog study [clinical signs of emesis, lacrimation, salivation and tremors and cholinesterase inhibition (ChEI)], mouse carcinogenicity study (ChEI), rat combined chronic toxicity/carcinogenicity study (ChEI) and the rabbit developmental toxicity study (ChEI).

Acute Neurotoxicity Study in Rats

Executive Summary: In an acute neurotoxicity study (MRID # 43845204), groups of 12 male and 12 female Sprague-Dawley rats were administered carbaryl technical grade in 0.5% carboxymethylcellulose / 0.1% Tween 80 at doses of 10, 50, or 125 mg/kg/day. Doses were selected on the basis of results from a benchmark toxicity study (MRID # 43845201) and a "time of peak effects" study (MRID # 43845202). In the benchmark study, clinical signs of toxicity and body weight loss were observed at 50 mg/kg and above, and mortality was observed at 500 mg/kg and above. In the time of peak effects study, peak effect for cholinesterase inhibition and functional observational battery changes was determined to be 0.5 to 1.0 hr post-dose. Body weight was mildly but significantly decreased in male rats at the 125 mg/kg dose level, while weight gain was significantly decreased in male and female rats for days 0-7 of the study at 125 mg/kg. Food consumption during week 1 was decreased at the 125 mg/kg dose by 18-20%, in excess of the decrease in body weight gain, supporting a treatment-related effect at the high dose

for week 1 of the study. Several measurements from Functional Observational Battery assessment were significantly altered at the 50 and 125 mg/kg dose, including an increased incidence of tremors, ataxic gait, decreased body temperature, and decreased arousal. Salivation incidence was increased at the high dose, as was hindlimb splay. Forelimb and hindlimb grip strength were decreased significantly at the high dose. Significant decreases in total motor activity were observed in male and female rats at all dose levels tested. Significant inhibition of plasma, blood, and brain cholinesterase (30-40%) was also observed in both sexes at the 10 mg/kg dose. Peak inhibition of cholinesterase occurred during the time of FOB and motor activity measurements. Based on the data in this study, the systemic LOAEL = 10 mg/kg for male and female rats, based on significant inhibition of red cell, plasma, whole blood, and brain cholinesterase at the 10 mg/kg dose level. The systemic NOAEL < 10 mg/kg for male and female rats. This study is classified as acceptable and satisfies the guideline requirement for an acute neurotoxicity study (§81-8; OPPTS 870.6200) in rats.

Subchronic Neurotoxicity Study in Rats

Executive Summary: In a subchronic neurotoxicity study, 12 Crl:CD(SD)BR rats/sex/group were administered technical carbaryl (99.1%) by gavage at doses of 0, 1, 10 or 30 mg/kg/day for 13 weeks. Cholinesterase (RBC, whole blood, plasma and brain) determinations were done on an additional three groups of five rats/sex/group at Weeks 4, 8 and 13. Neurobehavioral screening, consisting of Functional Observational Battery (FOB) and motor activity evaluations, was performed prior to treatment and during Weeks 4, 8 and 13. At terminal sacrifice, six animals/sex/dose were anesthetized and perfusion fixed *in situ* for neuropathological evaluation.

There were no deaths during the study. There was an increased incidence of clinical signs of toxicity, including slight and moderate salivation and tremors, in the 30 mg/kg/day males and females. Body weight over the course of the study was statistically significantly decreased in the 30 mg/kg/day males (14%) and females (15%). Body weight gain for these groups was decreased 27% in males and 37% in females, compared to controls. Food consumption was decreased during most of the study for the 30 mg/kg/day males and females. Males and females in the 30 mg/kg/day group had a statistically significant decrease in RBC (M:42-46%; F:52-55%), whole blood (M: 49-51%; F: 59-63%) and plasma (M: 63-69%; F: 63-69%) at most of the testing periods. Males and females in the 10 mg/kg/day group had a statistically significant decrease in RBC (M: 26-38%; F: 17-24%); whole blood (M: 30-41%; F: 21-26%) and plasma (M:43-48%; F: 23-30%). There was a statistically significant decrease in brain cholinesterase in males and females in the 10 mg/kg/day (M: 27-61%; F: 20-58%) and 30 mg/kg/day (M: 36-80%; F: 50-73%) groups. For the 1 mg/kg/day males, there were statistically significant decreases in whole blood (13%) at week 13 and for plasma (20%) at week 8. These changes are not considered toxicologically significant since they occurred infrequently and were relatively minor effects.

Multiple qualitative and quantitative FOB parameters were affected in the 10 and 30 mg/kg/day males and females, including the following: slight tremors, gait alterations, pinpoint pupils, increased salivation, reduced extensor thrust, decreased pinna reflex, reduced number of rearings, decreased vocalizations, decreased body temperature and decreased forelimb grip.

Reduced number of defecations was observed only at 30 mg/kg/day. There was an occasional alteration at the 1 mg/kg/day dose. At week 8, males had a very slight increase in the incidence of pinpoint pupils (incidence in control, 1, 10 and 30 mg/kg/day groups was 0/12, 1/12, 6/12 and 10/12, respectively). A statistically significant decrease in forelimb grip was observed at week 4 in males (values for control, 1, 10 and 30 mg/kg/day groups were 1060.8, 943.8, 943.8 and 950.0, respectively). The number of defecations was statistically reduced in females at week 13 (mean number of defecations in control, 1, 10 and 30 mg/kg/day groups were 1.4, 0.2, 0.5 and 0.0, respectively). The toxicological significance of these effects is questionable since the incidence was either low or there was no dose-response relationship.

Motor activity was statistically significantly decreased in the 30 mg/kg/day males at Week 4 and the 30 mg/kg/day females at Weeks 4 and 8.

On necropsy, there was an increased incidence of dark areas in the meninges of the 30 mg/kg/day males; these animals had an increased incidence of hemorrhage on microscopic examination. One female in the 30 mg/kg/day group also had retinal atrophy. There were no differences in brain length or width measurements.

The LOAEL for neurotoxicity was 10.0 mg/kg/day based on an increased incidence of FOB changes; the NOAEL was 1.0 mg/kg/day. The LOAEL for cholinesterase inhibition was 10.0 mg/kg/day based on statistically significant decreases in RBC, whole blood, plasma and brain cholinesterase; the NOAEL was 1.0 mg/kg/day.

Developmental Neurotoxicity Study in Rats

Executive Summary: In a developmental neurotoxicity study (MRID # 44393701, 45456701, 45456702, 45456703), 26 pregnant female Sprague-Dawley rats/group were administered carbaryl (99.1% a.i.) by gavage from Gestation Day (GD) 6 through Lactation Day (LD) 10 at doses of either 0, 0.1, 1.0 or 10 mg/kg/day. An additional 6 pregnant females/group were dosed at the same levels for the cholinesterase (ChE) phase of the study. ChE measurements were done pre-dosing (GD 6) and post-dosing at time of peak effect (1 hour post-dosing) on GD 6, 15 and 20 and LD 4 and 10. Functional Observational Battery (FOB) measurements were performed at approximately 0.5 and 2 hours post-dosing on the same days as body weight measurements during the dosing period (GD 0, 6, 9, 12, 15, 18 and 20 and LD 4, 7, 11, 13 and 21). Measures of reproductive performance were evaluated. Offspring were examined for body weight, physical development landmarks (tooth eruption and eye opening), FOB assessments (days 4, 7, 11, 13, 17 and 21) and motor activity (days 13, 17 and 21). On LD 11, 1 animal/sex/litter was sacrificed for brain weights; of these, six/sex were randomly selected for neuropathological evaluation. The eyes from all dose groups were examined. After LD 21, 3 animals/sex/litter were separated from the dams and constituted the F1 adult generation. These animals were evaluated for body weight, physical development (vaginal opening and preputial separation), motor activity (day 60), startle habituation response (days 22 and 60), passive avoidance (day 23) and water maze behavior (day 60). After completion of the behavior test period (at approximately 10 weeks of age), 12 animals/sex/group were anesthetized and perfused for post-mortem examination. Tissues from 6 animals/sex of the control and high dose group were processed for neuropathological evaluation and morphometric measurements;

the eyes from the low and mid-dose group of all perfused animals were examined.

For the F0 generation animals, there were no carbaryl-associated deaths. No treatment-related clinical signs of toxicity were observed. There was a statistically significant decrease (92%) in body weight gain for females in the 10 mg/kg/day group for the period GD 6-9. Unfortunately, food consumption was not measured during the study. During the FOB measurements, the incidence of females in the 10 mg/kg/day group with decreased pupil size (pinpoint pupils) was increased on all occasions during the dosing period. An increased incidence of dams with slight tremors affecting the head, body and/or limbs was noted on the majority of assessment occasions in the dosing period. There were also occasional occurrences of ataxic gait/overall gait in-capacity which was considered to be of toxicological significance due to other effects upon gait.

For the 10 mg/kg/day group, RBC and whole blood ChE levels were statistically significantly decreased (28% and 32-34%, respectively) on GD 20 and LD 10. Although the plasma ChE levels were not statistically significantly altered, the percentage decreases on GD 20, LD 4 and

LD 10 were 32-39%. Brain ChE levels were statistically significantly decreased (42%). There were no treatment-related effects on gross necropsy findings for the F0 generation animals.

There were no effects observed on maternal performance parameters of pregnancy rate, gestation index, length of gestation, numbers of live pups, dead or malformed pups, implantation scars, sex ratio or post-implantation loss. There was a slight ($P>0.05$) increase in the number of dead pups in the 10 mg/kg/day group, however the value was within the historical control range for this strain.

For the F1 generation pups, there were no treatment-related effects on pup weight, pup survival indices, developmental landmarks (tooth eruption and eye opening), FOB measurements or motor activity assessments. At sacrifice on LD 11, there were no treatment-related effects on brain weight and gross or microscopic pathology. Significant differences noted in the morphometric measurements included an increase in Line B of the right forebrain and Line F of the left cerebellum in the 10 mg/kg/day males. In the 10 mg/kg/day females, Line F through both the right and left cerebellum were significantly decreased (15% and 22%, respectively).

For the F1 generation adults, there were no treatment-related effects on clinical condition, body weight, physical development (vaginal opening and preputial separation), motor activity, auditory startle response, passive avoidance and water maze measurements. At sacrifice, there were no gross or microscopic neuropathological lesions observed for animals examined in this study that were attributable to treatment with the test article. There was an increased incidence of retinal fold/rosette in the 10 mg/kg/day group (1/12 for control vs. 4/12 for males; 0/12 for control vs. 2/12 for females). The finding was not considered of toxicological significance since the incidence was within the historical control range for males, occurred at a low rate and was not dose-dependent. For the morphometric measurements, there was a significant bilateral decrease in Line A through the forebrain (7.7-9.8%) and a significant increase in Line F through the right cerebellum of the 10 mg/kg/day males. Increases originally noted in 10 mg/kg adult females in Line G, width of the cerebellum, were found to be based on erroneous

measurements, and additional measures were submitted. Now, for the 10 mg/kg/day females, there were significant bilateral increases in Line F through the cerebellum (7.4-15%). Measurements of the size of the thickness of lobes and of the granule cell layers of the cerebellum in high dose pups and adults did not differ from those of controls. While additional statistical analyses by the registrant indicated no treatment related effects, HED's additional statisical analyses did indicate treatment related effects.

The maternal toxicity LOAEL was 10 mg/kg/day based on decreased body weight gain, alterations in FOB measurements and RBC, plasma, whole blood and brain cholinesterase inhibition. The maternal NOAEL was 1.0 mg/kg/day.

The developmental neurotoxicity LOAEL was 10 mg/kg/day based on a bilateral decrease in the size of the forebrain (Line A) in adult males (7.7-9.8%); a bilateral decrease in the length of the cerebella (Line F) in female pups (15-22%); and a bilateral increase in the length of the cerebella (Line F) in female adults (7.4-15%).

The developmental NOAEL was 1 mg/kg/day. Morphometric assessment at the mid and low doses could not be conducted due to inadequate tissue storage; however, based on the minimal findings at the LOAEL, it is HED's judgment that effects would be unlikely to occur at 1 mg/kg/day, which is 10% of the LOAEL.

3. Developmental Toxicity Study Conclusions

Prenatal developmental toxicity study in the rat

In a developmental toxicity study (MRID 44732901), carbaryl (99% a.i.) in an aqueous methylcellulose suspension was administered by gavage at 0, 1, 4, and 30 mg/kg/day to pregnant Crl: CD (SD) BR rats (25/dose) during gestation days (GDs) 6 through 20. At GD 21, surviving dams were sacrificed and necropsied.

There were no treatment-related gross pathologic findings noted in any of the dams. There were no differences of toxicological concern in mortality, pregnancy rate, numbers of corpora lutea, implantations, viable fetuses, pre- and post-implantation losses, placental weights, and sex ratio.

At 30 mg/kg/day, at least one occurrence of post-dosing salivation occurred in 18/25 of the dams (vs 0/25 controls). This clinical sign appeared within 20 minutes of treatment, disappeared after approximately one hour, and was observed from GD 13 to 20. There were no deaths and no other treatment-related clinical signs. Body weights of the high-dose dams were 3-8% less than controls throughout the study (not statistically significant); their corrected (for gravid uterine weight) body weights and body weight gains were decreased ($p \leq 0.01$) by 7 and 38%, respectively. Body weight gains in this group were decreased immediately after initiation of dosing (GDs 6-9, $\downarrow$108%, $p \leq 0.01$) and throughout treatment (overall, $\downarrow$27%, $p \leq 0.01$). Food consumption (g/animal/day) was decreased throughout the treatment period ($\downarrow$10-17%, $p \leq 0.01$).

There were no differences of toxicological concern observed in the mid- and low-dose groups.

The maternal LOAEL is 30 mg/kg/day based on clinical signs of toxicity, decreased body weight gains and food consumption. The maternal NOAEL is 4 mg/kg/day.

In the high-dose fetuses, mean fetal body weights were reduced ($\downarrow$7-8%, $p \leq 0.01$). Additionally, the following were observed in the high-dose male and female fetuses: (i) an increase in incomplete ossification of the 5th sternebra, (ii) unossified 7th cervical centrum, (iii) incomplete ossification of 7th cervical centrum, and (iv) unossified 1st metatarsal. No effects on fetal viability were observed.

There were no treatment related effects in developmental parameters observed in the mid- and low-dose groups.

The developmental LOAEL is 30 mg/kg/day based on decreased fetal body weights and increased incomplete ossification of multiple bones. The developmental NOAEL is 4 mg/kg/day.

The developmental toxicity study in the rat is classified as acceptable (§83-3(a)) and does satisfy the guideline requirement for a developmental toxicity study in the rat.

Prenatal developmental toxicity study in the rabbit

In a developmental toxicity study (MRID 44904202), carbaryl (99% a.i.) in an aqueous methylcellulose suspension was administered by gavage at doses of 0, 5, 50 or 150 mg/kg/day to pregnant New Zealand White rabbits (22/dose) during Gestation Days (GD) 6-29. On GD 25, blood was collected 1 hour post-dosing for plasma and red blood cell (RBC) cholinesterase (ChE) measurements. At GD 30, surviving dams were sacrificed and necropsied; fetuses were examined for evidence of developmental effects. Maternal toxicity at 150 mg/kg/day was observed as statistically significant decreased body weight gain as compared to the control value during GD 6-9 (208%), GD 6-29 (dosing period, 53%), GD 3-30 (33%) and gestation (GD 0- GD 30, 38%). Corrected body weight change was also decreased at this dose (-219.73 g vs -81.86 g in the control). Although not statistically significant, the body weight decreases at 50 mg/kg/day can be considered biologically significant for GD 6-9 (55%), GD 6-29 (25%), GD 3-30 (14%) and gestation (14%). There was no treatment-related effect on food consumption. Statistically significantly decreases in plasma (46-68%) and RBC (19-27%) ChE were seen at 50 and 150 mg/kg/day.

Maternal LOAEL = 50 mg/kg/day based on decreased body weight gain and decreased plasma and RBC ChE; Maternal NOAEL = 5 mg/kg/day

The only evidence of developmental toxicity was a statistically significant decrease in fetal body weights of 10% (when calculated for all fetuses or individually for males and females) at 150 mg/kg/day. There were no treatment-related developmental effects observed in the mid- and low-dose groups.

Developmental Toxicity LOAEL is 150 mg/kg/day based on decreased fetal weight.
Developmental Toxicity NOAEL is 50 mg/kg/day

The developmental toxicity study in the rabbit is classified as acceptable/guideline and does satisfy the guideline requirement for a developmental toxicity study in the rabbit.

4. Reproductive Toxicity Study Conclusions

Reproductive Toxicity in Rats

In a two-generation reproduction study (MRID 45448101), carbaryl (99.1% a.i, Lot No. E1208008) was given in the diet to groups of 30 male and 30 female F_0 and F_1 rats (CD®[SD] IGS BR (Sprague-Dawley)) at concentrations of 0, 75, 300, or 1500 ppm. The dietary concentrations corresponded to doses of 4.67, 31.34, and 92.43 mg/kg/day for F_0 males; 0, 5.56, 36.32, and 110.78 mg/kg/day for F_0 females; 0, 5.79, 23.49, and 124.33 mg/kg/day for F_1 males; and 0, 6.41, 26.91, and 135.54 mg/kg/day for F_1 females averaged over the premating period. Each group received treated or control diet continuously for 70 days prior to mating and during mating, gestation, and lactation of one litter per generation. F_1 pups selected to parent the F_2 generation were weaned onto the same food as their parents. Parental males were sacrificed after delivery of their litters and parental females were sacrificed after weaning of their litters.

No treatment-related deaths, clinical signs, organ weight changes, gross lesions, or microscopic lesions were observed in adult rats of either generation. No treatment-related effects were observed on body weights, weight gain, feed consumption, or food efficiency in 75- or 300-ppm group F_0 or F_1 male or female rats at any time during the study including the gestation and lactation periods of the females. F_0 and F_1 male and female rats fed the 1500-ppm diet weighed significantly ($p<0.01$ or <0.05) less and gained less weight during the premating period. The F_0 males weighed 5-6% less than controls during premating, gained 14-23% less weight during three weekly intervals up to day 45, and gained 9% less weight over the entire premating period; they also gained 8% less weight than controls over the mating/postmating period. The F_1 males weighed 10-19% less than controls during the entire study, gained 16% and 11% less weight during the first two weekly intervals, and gained 8% less weight than controls averaged over the entire premating period. The F_0 females weighed 4-5% less than controls during the first 42 days of premating, gained 27% less weight during the first week, and 7% (N.S.) less averaged over the entire premating period. The F_1 females weighed 8-22% less than controls throughout premating and gained 9% less weight during the first week; weight gain for the remaining weekly intervals and for the entire premating period was similar to that of controls. Food consumption and food efficiency for F_0 and F_1 rats followed patterns similar to that of body weight and weight gain; the largest difference between the 1500-ppm groups and controls occurred during the early part of the premating period. When averaged over the entire premating period, F_0 and F_1 males consumed 6-7% less food than control and had food efficiency values similar to those of the controls. Feed consumption and food efficiency for the F_0 females were similar to those of the control group, whereas F_1 females consumed 9% ($p<0.01$) less feed and had a food efficiency value 10% ($p<0.01$) greater than

that of controls. F_0 and F_1 females in the 1500 ppm group weighed less and gained less weight than controls during gestation, with the effect being greater in the F_1 females. During lactation weight gain was markedly reduced in F_1 females during the first 4 days, but was greater than that of controls averaged over the entire lactation period.

The lowest-observed-effect level (LOAEL) for parental systemic toxicity is 1500 ppm (92.43-124.33 mg/kg/day for males and 110.78-135.54 mg/kg/day for females) based on decreased body weight, weight gain, and feed consumption. The no-observed-adverse-effect (NOAEL) level is 300 ppm (23.49-31.34 mg/kg/day for males and 26.91-36.32 mg/kg/day for females).

No treatment-related effects were observed on the estrous cycle of either F_0 or F_1 females at any dose level or on percent motile sperm, sperm count, percent progressively motile sperm, epididymal sperm count, spermatid head count, daily sperm production, or efficiency of daily sperm production in F_0 or F_1 males at any dose level. There was a dose-related increase in the percentage of abnormal sperm in the treated males but no statistical significance at any dose level. No treatment-related gross or microscopic effects were observed in male or female rats of either generation. No treatment-related effects were observed on any parameter of reproductive performance including, mating and fertility indexes, gestation index, pregnancy index, precoital duration, gestation length, or number of females producing live litters.

The LOAEL for reproductive toxicity could not be established because no effects were observed at any dose level; therefore, the NOAEL is $\geq$1500 ppm (92.43-124.33 mg/kg/day for males and 110.78-135.54 mg/kg/day for females).

No treatment-related effects were observed on implantation sites/litter, number of live pups born/litter, number of dead pups born/litter, live birth index, sex ratio, clinical signs, or organ weight or necropsy findings in pups surviving to 21 days. Pup survival was decreased at 300 and 1500 ppm for both generations. Increased number of deaths in the F_2 generation males and females resulted in an 18-19% decrease in mean litter size on postnatal day 4 ($p<0.01$ or <0.05) and decreased viability and lactation indexes at 1500 ppm. A large number of pups that died had no milk in their stomachs. In addition, pup weight/litter and pup weight gain in the 1500-ppm group pups were reduced for both generations starting with postnatal day 4 (11-15% for F_1 and 13-23% for F_2 pups); body weight gain was reduced throughout lactation with the greatest effect occurring during the first 7 days for F_1 pups and the first 14 days for F_2 pups. Sexual maturation was delayed in 1500-ppm group F_1 offspring as evidenced by delayed balanopreputial separation in the males (+2.1 days) and vaginal patency in the females (+1.4 days). The differences remained statistically significant after adjustment for body weight decreases. Anogenital distance was significantly reduced in F_2 male pups in the 1500-ppm group, but not when the distance was adjusted for body weight.

The LOAEL for offspring toxicity was 300 ppm (23.49-31.34 mg/kg/day for males and 26.91-36.32 mg/kg/day for females) based on increased numbers of F_2 pups with no milk in the stomach and decreased pup survival. The NOAEL is 75 ppm (4.67-5.79 mg/kg/day for males and 5.56-6.41 mg/kg/day for females).

This study is **Acceptable/Guideline** and satisfies the guideline requirement for a two-generation reproductive study (OPPTS 870.3800; OECD 416) in the rat.

5. <u>Additional Information from Literature Sources</u>

In an unpublished study from the National Health and Ecological Effects Research Laboratories, EPA, and the National Institute for Environmental Health Sciences/National Toxicology Program, pregnant Sprague-Dawley rats (n=36 or 38) were dosed by gavage with carbaryl at doses of 0, 6, 12 or 25 mg/kg/day.[1] The following description of the study design and findings was extracted from tables and posters discussing various aspects of the study. The dams were dosed from gestational day (GD) 14 to postnatal day (PND) 7, after which the pups were directly dosed with the same dose levels until PND 21 (weaning) or PND 42. Analyses for carbaryl and 1-naphthol in the dam's plasma and milk and pup's plasma were performed. A sample of milk was incubated with a preparation of rat brain to provide a bioassay of ChE activity. The brains were taken from a dam and two fetuses sacrificed at various times after dosing on GD 18 to measure ChE. Some pups (n=4-6/dose/sex) were sacrificed on PND 1, 7, 21 and 47 and body and brain weights recorded. FOB and motor activity were measured on PND 26/27, 47/48, 62/63 and 81/82. In the post-weaning period, cognitive function was evaluated using a simple test of associative learning, passive avoidance, and in adulthood by assessing between-session habituation of motor activity. Sperm counts, organ weights and clinical pathology were done on males at necropsy. Carbaryl or 1-naphthol were not present in the pups' plasma above the limit of detection at any exposure concentration. In the dams' plasma, carbaryl was below the limit of detection for the 6 mg/kg/day dose, but was present in some or most of the animals from the other two doses. 1-naphthol was present in all treated groups in a dose-related increase. In general, milk concentrations followed the trends seen in plasma, however 1-napthol was about 3-5 times lower in milk compared to plasma. There was a dose-related suppression of brain ChE produced by the blood samples. There was a dose-related decrease in ChE activity in the brain and blood of dams at GD 19, and fetuses taken at that time also showed a very similar level of inhibition in fetal brain. There was a decrease in the number of live pups/litter in the 25 mg/kg/day group at PND 0, 7, and 21. The average pup weight was decreased in the 25 mg/kg/day group at PND 1, 7, 14 and 21. There were no changes in cognitive function. For brain weights measured on PND 0, 7, 21 and 47, the only change was on PND 21 when the 25 mg/kg/day group was decreased in males and the low and high dose groups were decreased in females. Equivocal changes in FOB parameters were observed in males at PND62/63 and in females at PND 47/48. There were no evidence of an effect on the necropsy parameters.

In a 1996 study in the open literature, carbaryl was administered to four groups of 6 young and 6 adult Druckery albino rats per group at doses of 0, 25, 50 or 100 mg/kg/day for 60 days.[2] Body weight was recorded at initiation and completion of the study. On the 61st day, the animals were sacrificed and the testes, epididymides, seminal vesicles, ventral prostrate and

[1] Personal communication with Robert Chapin, one of the study authors

[2] Pant N, Shankar R, Srivastava SP (1996). Spermatotoxic effects of carbaryl in rats. Human Exp Toxicol 15(9); 736-38.

coagulating glands were weighed. Epididymal sperm were used for sperm counts and examination of motility and morphology. No overt toxicity or mortality was observed. There were dose-related effects on body weight for the 50 and 100 mg/kg/day groups. The absolute weights of the testes, epididymides, seminal vesicle, ventral prostrate and coagulating glands were significantly decreased at 100 mg/kg/day for young rats. The relative organ weights were not affected at any doses. The organ weights were not affected in adult animals. Young rats receiving carbaryl 50 mg/kg/day had a 24.4% and 25% decrease in sperm motility and sperm count, respectively; the changes at 100 mg/kg/day were 42.9% and 37.5%, respectively. Adults receiving the 50 mg/kg/day dose had a 15.1% and 12.5% reduction in sperm motility and count, respectively; the changes at 100 mg/kg/day were 26.4% and 25%, respectively. The percentage of young rats with abnormal sperm was 19.8% and 33.7% at 50 and 100 mg/kg/day, respectively. In adults, the percentages were 16.1% and 23.1% for the respective doses.

In another study from this laboratory, three groups of 8 male Wistar rats per group were administered carbaryl by gavage at doses of 0, 50 or 100 mg/kg/day for 90 days.[3] Body weight was measured periodically throughout the study. On the 91st day, the animals were sacrificed and the male reproductive glands were weighed. One testis from each animal was preserved for histopathology and the other was homogenized for testicular enzyme assay. Epididymal sperm were used for sperm counts and examination of motility and morphology. No clinical signs of toxicity were observed, except for lethargy. Body weights were decreased in the 100 mg/kg/day group after 60 days. There were no changes in the weights of reproductive organs. There were significant changes in the testicular enzymes of the 100 mg/kg/day group: decreases in SDH and G6PDH and increases in GGT and LDH. At both doses, there were significant decreases in the total epididymal sperm count, percent sperm motility and increases in the percent with morphological abnormalities in head, neck and tail. At 50 mg/kg/day, the testes had slight to moderate congestion and edema. A few tubules showed moderately depressed spermatogenesis and loss of sperm. There was moderate atrophy of seminiferous tubules with prominent interstitial spaces in the center of the testes, but the Leydig cells were intact. At 100 mg/kg/day, there were increases in the intensity of congestion and the edematous reaction was seen both peripherally and in the central region. Most of the seminiferous tubules had disturbed spermatogenesis as well as accumulations of cellular masses in their lumens.

In a study conducted at EPA's Health Effects Research Laboratory, 16 pregnant Fischer 344 rats were administered carbaryl by gavage on gestation days (GD) 6-19 at doses of 78 or 104 mg/kg/day; 21 control animals were used.[4] The high dose, selected to produce overt maternal toxicity, was based on the results of a 14-day repeated dose study in nonpregnant female rats. The low dose was 75% of the high dose. Maternal body weights were determined on GD 6, 8, 10, 13, 16 and 20. All rats were examined periodically for clinical signs of toxicity. Pups in each litter were examined and counted on postnatal day (PD) 1, 3, and 6 and weighed collectively on PD 1 and 6. After the final litter examination, the dams were killed and uterine

[3] Pant N, Srivastava SC, Prasad AK, Shankar R, Srivastava SP (1995). Effects of Carbaryl on the Rat's Male Reproductive System. Vet Human Toxicol 37(5): 421-425.

[4] Narotsky MG, Kavlock RJ (1995). A Multidisciplinary Approach to Toxicological Screening: II. Developmental Toxicity. Journal of Toxicology and Environmental Health 45:145-171.

implantation sites counted. Females that did not deliver by GD 24 were killed and their uteri examined for pregnancy status. Clinical signs of toxicity observed in the dams included tremors, motor depression, and lacrimation, usually during the first three days of treatment. Jaw clonus was observed throughout the treatment period. (The article does not indicate if clinical signs were observed at both doses.) Marked weight loss was observed early in treatment. Over the entire treatment period, carbaryl produced extrauterine weight loss at the high dose and reduced weight gains at the low dose. There was increased prenatal mortality at the high dose; this effect was attributed to two (15%) fully resorbed litters in this group. In addition, high dose pup weights were significantly reduced on PD 1. The PD-1 pup weights in the low dose and the PD 6 pup weights in both carbaryl-exposed groups were also

significantly reduced, but only when analyzed using the number of live pups on PD 1 as the covariate.

In a recent epidemiology study, the effects of exposure of male farmers in Ontario, Canada, to agricultural pesticides and pregnancy outcome was investigated.[5] Miscarriage risk was not associated with participation in farm activities for all types of chemical applications, but was increased in combination with reported use of thiocarbamates, carbaryl and unclassified pesticides on the farm (Odds ratio = 1.9, 95% C.I. 1.1-3.1). There was no association between use of carbaryl and preterm delivery, small for gestational age or altered sex ratio measurements.

At the 1996 Joint Meeting on Pesticide Residues (JMPR), it was concluded that carbaryl induces developmental toxicity, manifested as deaths *in utero,* reduced fetal weight, and malformations, but only at doses that cause overt maternal toxicity. The shortcomings of the developmental studies made them inadequate for identifying NOAELs for developmental toxicity that could be used for assessing risk under conditions of exposure other than in the diet. The Committee recommended studies of teratogenicity in rats and rabbits and study of developmental neurotoxicity and/or screening for acute or subchronic neurotoxicity. Two dog studies were cited in the report. In these studies, maternal toxicity (dystocia, at parturition only) was observed at a dose of 3.1 mg/kg/day. Various birth defects were observed in the pups at doses $\geq$ 5 mg/kg/day. Thus the LOAEL for maternal toxicity was 3.1 mg/kg/day, which was the NOAEL for birth defects in the offspring.

The report states that studies on reproductive toxicity were conducted some time ago and had some deficiencies in relation to currently acceptable scientific standards. The Meeting recommended that a new two-generation reproductive toxicity study should be carried out on rats, with special attention to the male reproductive system since effects on this system were observed in some long-term studies of toxicity at gavage doses significantly lower than those evaluated in the dietary studies of reproductive toxicity.

6. Pre-and/or Postnatal Toxicity

[5] Savitz DA, Arbuckle T, Kaczor D, Curtis KM (1997). Male Pesticide Exposure and Pregnancy Outcome. Am J Epidemiol 146(12):1025-36.

The HIARC concluded that there is a concern for pre- and/or postnatal toxicity resulting from exposure to Carbaryl.

A. Determination of Susceptibility

There was no evidence of quantitative or qualitative susceptibility following *in utero* exposures in developmental studies in the rat and rabbit.

In the reproduction study, there was evidence of quantitative susceptibility of offsprings. The LOAEL for parental systemic toxicity was based on decreased body weight, weight gain, and feed consumption; the NOAEL was 27 mg/kg/day in males and 30 mg/kg/day in females. In the offspring the LOAEL was based on increased numbers of F_2 pups with no milk in the stomach and decreased pup survival; the NOAEL was 5 mg/kg/day in males and 6 mg/kg/day in females. No adverse effects were observed in the reproductive parameters; the NOAEL was the highest dose tested.

In the developmental neurotoxicity study, there was evidence of qualitative susceptibility. For maternal toxicity, the LOAEL was based on decreased body weight gain, alterations in Functional Observational Battery measurements and inhibition of plasma, whole blood and brain cholinesterase activity; the NOAEL was 1 mg/kg/day. For developmental neurotoxicity, the LOAEL was based on the morphometric changes seen in the brain of the offsprings; the NOAEL was 1 mg/kg/day.

B. Degree of Concern Analysis and Residual Uncertainties

Since there is evidence of increased susceptibility of the young following exposure to Carbaryl in the 2-generation reproduction study and in the developmental neurotoxicity study, HIARC performed a Degree of Concern Analysis to: 1) determine the level of concern for the effects observed when considered in the context of all available toxicity data; and 2) identify any residual concerns after establishing toxicity endpoints and traditional uncertainty factors to be used in the risk assessment of this chemical. If residual concerns are identified, HIARC examines whether these residual concerns can be addressed by a special FQPA safety factor and, if so, the size of the factor needed. The results of the HIARC Degree of Concern analyses for Carbaryl follow.

A. 2-Generation Reproduction Study

The HIARC concluded that there are no residual concerns related to the 2-generation reproduction study because the dose-response for the offspring effects is well-characterized and these effects occurred at a dose level which is above that used for establishing the Chronic Reference Dose (cRfD) for chronic dietary risk assessment.

The HIARC established the Chronic RfD using the LOAEL of 3.1 mg/kg/day in the chronic toxicity study in dogs. Since a NOAEL was not established in this study, an additional uncertainty factor of 3X was applied to the LOAEL (i.e, UF_L). The HIARC determined that 3X is adequate to account for the lack of a NOAEL in this case because: 1) the study was well-

conducted and there are sufficient data from subchronic and other chronic studies in other species that support cholinesterase inhibition as the critical effect for Carbaryl; 2) the data indicate that the dog is more sensitive to the cholinergic effects of Carbaryl and using this species to establish the RfD provides additional protection for the effects seen in the rat (including the reproduction and developmental neurotoxicity studies); 3) the magnitude of plasma cholinesterase inhibition (12-23% decrease) seen in this study was comparable to the magnitude of inhibition (22%) seen in the 5-week study in dogs - indicating no cumulative effect following long-term exposure; 4) the cholinesterase inhibition seen in females at the LOAEL in this study was not accompanied by clinical signs (response was not judged to be severe); and 5) no inhibition was seen for any cholinesterase compartment in males at this dose (response was seen in only one sex).

The HIARC concluded that the extrapolated NOAEL of 1 mg/kg/day used to establish the Chronic RfD for Carbaryl is below the NOAEL for offspring toxicity (5 mg/kg/day) in the 2-generation reproduction study and is protective of chronic dietary exposures to infants and children.

B. Developmental Neurotoxicity Study

The HIARC concluded that there was a low level of concern for the developmental effects seen in the developmental neurotoxicity study and no residual uncertainties with respect to this study based on the following evidence:

- Any concern for the lack of brain morphometric measurements in the offspring at the mid-dose (1 mg/kg/day) was negated since even at the high dose of 10 mg/kg/day, the morphometric changes were minimal and therefore, it is unlikely that adverse effects would be seen at the mid-dose level (1 mg/kg/day - 10% of the LOAEL).

- Any concern for the lack of comparative data in adults and offspring for cholinesterase inhibition was negated since no FOB alterations were seen in pups. Other studies in the data base show that when cholinesterase inhibition was seen in adult animals, it usually was accompanied by FOB alterations. Additionally, the results of the National Institute for Environmental Health Sciences study (discussed above) indicate that there is no difference in cholinesterase inhibition in pups and adults. The dose-related decrease in cholinesterase activity in the brain and blood of dams at gestation day 19 was very similar to the fetal brain cholinesterase levels taken at the same time.

The HIARC established the Acute RfD for Carbaryl using the NOAEL of 1 mg/kg/day in the developmental neurotoxicity study in rats which is protective of single dose exposures to infants and children.

4. Summary of Open Literature Findings

In the scientific literature, there are two relatively recent studies which demonstrated effects on sperm at high doses (50 and 100 mg/kg/day) of Carbaryl. The results of these two studies

indicated that Carbaryl caused weight reductions in the testes, epididymides, seminal vesicles, prostate and coagulating glands of young rats; changes in testicular enzymes; decreased sperm counts and sperm motility; increased sperm morphological abnormalities; and moderate atrophy of seminiferous tubules of the testes.

In a published developmental study in Fisher 344 rats conducted by EPA's Health Effects Research Laboratory, Carbaryl was administered from gestation day 6 through 19 at doses of 78 or 104 mg/kg/day. Clinical signs related to cholinesterase inhibition (tremor, motor depression, jaw clonus and lacrimation) were observed in dams but it is unclear if they occurred at both dose levels. There was also increased prenatal mortality at the high dose (104 mg/kg/day) and decreased pup weights at the low (78 mg/kg/day) doses.

In an unpublished developmental neurotoxicity study in SD rats from the National Health and Ecological Effects Research Laboratories at EPA and the National Institute for Environmental Health Sciences/National Toxicology Program Carbaryl was administered by gavage at doses of 0, 6, 12 or 25 mg/kg/day. The chemical or its metabolite 1-naphthol was not present in pups' plasma above the limit of detection at any exposure concentration (0, 6, 12 or 25 mg/kg/day). There was a dose-related decrease in ChE activity in the brain and blood of dams at GD 19, and fetuses taken at that time also showed a very similar level of inhibition in fetal brain cholinesterase. There was a decrease in the number of live pups/litter at the high dose. There were no changes in cognitive function. Equivocal changes in Functional Observational Battery parameters were observed in male and female offspring.

II. HAZARD IDENTIFICATION

(Endpoints selected at the February 19, 2002 meeting are included for completeness.)

1. Acute Reference Dose (aRfD) - General Population

Study Selected: Developmental Neurotoxicity Study in Rats §81-8; OPPTS 870.6300

MRID Nos.: 44393701, 45456701, 45456702, 45456703

Executive Summary: In a developmental neurotoxicity study (MRID # 44393701, 45456701, 45456702, 45456703), 26 pregnant female Sprague-Dawley rats/group were administered carbaryl (99.1% a.i.) by gavage from Gestation Day (GD) 6 through Lactation Day (LD) 10 at doses of either 0, 0.1, 1.0 or 10 mg/kg/day. An additional 6 pregnant females/group were dosed at the same levels for the cholinesterase (ChE) phase of the study. ChE measurements were done pre-dosing (GD 6) and post-dosing at time of peak effect (1 hour post-dosing) on GD 6, 15 and 20 and LD 4 and 10. Functional Observational Battery (FOB) measurements were performed at approximately 0.5 and 2 hours post-dosing on the same days as body weight measurements during the dosing period (GD 0, 6, 9, 12, 15, 18 and 20 and LD 4, 7, 11, 13 and 21). Measures of reproductive performance were evaluated. Offspring were examined for body weight, physical development landmarks (tooth eruption and eye opening), FOB assessments (days 4, 7, 11, 13, 17 and 21) and motor activity (days 13, 17 and 21). On LD 11, 1 animal/sex/litter was sacrificed for brain weights; of these, six/sex were randomly selected for

neuropathological evaluation. The eyes from all dose groups were examined. After LD 21, 3 animals/sex/litter were separated from the dams and constituted the F1 adult generation. These animals were evaluated for body weight, physical development (vaginal opening and preputial separation), motor activity (day 60), startle habituation response (days 22 and 60), passive avoidance (day 23) and water maze behavior (day 60). After completion of the behavior test period (at approximately 10 weeks of age), 12 animals/sex/group were anesthetized and perfused for post-mortem examination. Tissues from 6 animals/sex of the control and high dose group were processed for neuropathological evaluation and morphometric measurements; the eyes from the low and mid-dose group of all perfused animals were examined.

For the F0 generation animals, there were no carbaryl-associated deaths. No treatment-related clinical signs of toxicity were observed. There was a statistically significant decrease (92%) in body weight gain for females in the 10 mg/kg/day group for the period GD 6-9. Unfortunately, food consumption was not measured during the study. During the FOB measurements, the incidence of females in the 10 mg/kg/day group with decreased pupil size (pinpoint pupils) was increased on all occasions during the dosing period. An increased incidence of dams with slight tremors affecting the head, body and/or limbs was noted on the majority of assessment occasions in the dosing period. There were also occasional occurrences of ataxic gait/overall gait in-capacity which was considered to be of toxicological significance due to other effects upon gait.

For the 10 mg/kg/day group, RBC and whole blood ChE levels were statistically significantly decreased (28% and 32-34%, respectively) on GD 20 and LD 10. Although the plasma ChE levels were not statistically significantly altered, the percentage decreases on GD 20, LD 4 and

LD 10 were 32-39%. Brain ChE levels were statistically significantly decreased (42%). There were no treatment-related effects on gross necropsy findings for the F0 generation animals.

There were no effects observed on maternal performance parameters of pregnancy rate, gestation index, length of gestation, numbers of live pups, dead or malformed pups, implantation scars, sex ratio or post-implantation loss. There was a slight ($P>0.05$) increase in the number of dead pups in the 10 mg/kg/day group, however the value was within the historical control range for this strain.

For the F1 generation pups, there were no treatment-related effects on pup weight, pup survival indices, developmental landmarks (tooth eruption and eye opening), FOB measurements or motor activity assessments. At sacrifice on LD 11, there were no treatment-related effects on brain weight and gross or microscopic pathology. Significant differences noted in the morphometric measurements included an increase in Line B of the right forebrain and Line F of the left cerebellum in the 10 mg/kg/day males. In the 10 mg/kg/day females, Line F through both the right and left cerebellum were significantly decreased (15% and 22%, respectively).

For the F1 generation adults, there were no treatment-related effects on clinical condition, body weight, physical development (vaginal opening and preputial separation), motor activity, auditory startle response, passive avoidance and water maze measurements. At sacrifice, there

were no gross or microscopic neuropathological lesions observed for animals examined in this study that were attributable to treatment with the test article. There was an increased incidence of retinal fold/rosette in the 10 mg/kg/day group (1/12 for control vs. 4/12 for males; 0/12 for control vs. 2/12 for females). The finding was not considered of toxicological significance since the incidence was within the historical control range for males, occurred at a low rate and was not dose-dependent. For the morphometric measurements, there was a significant bilateral decrease in Line A through the forebrain (7.7-9.8%) and a significant increase in Line F through the right cerebellum of the 10 mg/kg/day males. Increases originally noted in 10 mg/kg adult females in Line G, width of the cerebellum, were found to be based on erroneous measurements, and additional measures were submitted. Now, for the 10 mg/kg/day females, there were significant bilateral increases in Line F through the cerebellum (7.4-15%). Measurements of the size of the thickness of lobes and of the granule cell layers of the cerebellum in high dose pups and adults did not differ from those of controls. While additional statistical analyses by the registrant indicated no treatment related effects, HED's additional statisical analyses did indicate treatment related effects.

The maternal toxicity LOAEL was 10 mg/kg/day based on decreased body weight gain, alterations in FOB measurements and RBC, plasma, whole blood and brain cholinesterase inhibition. The maternal NOAEL was 1.0 mg/kg/day.

The developmental neurotoxicity LOAEL was 10 mg/kg/day based on a bilateral decrease in the size of the forebrain (Line A) in adult males (7.7-9.8%); a bilateral decrease in the length of the cerebella (Line F) in female pups (15-22%); and a bilateral increase in the length of the cerebella (Line F) in female adults (7.4-15%).

The developmental NOAEL was 1 mg/kg/day. Morphometric assessment at the mid and low doses could not be conducted due to inadequate tissue storage; however, based on the minimal findings at the LOAEL, it is HED's judgment that effects would be unlikely to occur at 1 mg/kg/day, which is 10% of the LOAEL.

Co-critical Study:

Study Selected: Acute Neurotoxicity Study in Rats §81-8; OPPTS 870.6200a

MRID Nos.: 43845201-43845204

Executive Summary: In an acute neurotoxicity study (MRID # 43845204), groups of 12 male and 12 female Sprague-Dawley rats were administered carbaryl technical grade in 0.5% carboxymethylcellulose / 0.1% Tween 80 at doses of 10, 50, or 125 mg/kg/day. Doses were selected on the basis of results from a benchmark toxicity study (MRID # 43845201) and a "time of peak effects" study (MRID # 43845202). In the benchmark study, clinical signs of toxicity and body weight loss were observed at 50 mg/kg and above, and mortality was observed at 500 mg/kg and above. In the time of peak effects study, peak effect for cholinesterase inhibition and functional observational battery changes was determined to be 0.5 to 1.0 hr post-dose. Body weight was mildly but significantly decreased in male rats at the

125 mg/kg dose level, while weight gain was significantly decreased in male and female rats for days 0-7 of the study at 125 mg/kg. Food consumption during week 1 was decreased at the 125 mg/kg dose by 18-20%, in excess of the decrease in body weight gain, supporting a treatment-related effect at the high dose for week 1 of the study. Several measurements from Functional Observational Battery assessment were significantly altered at the 50 and 125 mg/kg dose, including an increased incidence of tremors, ataxic gait, decreased body temperature, and decreased arousal. Salivation incidence was increased at the high dose, as was hindlimb splay. Forelimb and hindlimb grip strength were decreased significantly at the high dose. Significant decreases in total motor activity were observed in male and female rats at all dose levels tested. Significant inhibition of plasma, blood, and brain cholinesterase (30-40%) was also observed in both sexes at the 10 mg/kg dose. Peak inhibition of cholinesterase occurred during the time of FOB and motor activity measurements. Based on the data in this study, the systemic LOAEL = 10 mg/kg for male and female rats, based on significant inhibition of red cell, plasma, whole blood, and brain cholinesterase at the 10 mg/kg dose level. The systemic NOAEL < 10 mg/kg for male and female rats. This study is classified as **acceptable** and satisfies the guideline requirement for an acute neurotoxicity study (§81-8; OPPTS 870.6200) in rats.

Dose and Endpoint for Establishing RfD: Maternal NOAEL of 1 mg/kg/day based on alterations in FOB parameters on the first day of dosing at 10 mg/kg

Uncertainty Factor (UF): 100 [10 for intraspecies variation and 10 for interspecies variation].

Acute RfD =	$\frac{\text{1 mg/kg/day (NOAEL)}}{\text{100 (UF)}}$	=	**0.01**	**mg/kg**

Comments about Study/Endpoint/Uncertainty Factor: Previously (March 1, 2001), the HIARC selected the acute neurotoxicity study this risk assessment. However, upon reevaluation and comparison of the results of the acute neurotoxicity and the developmental neurotoxicity studies, the HIARC determined that the maternal effects in the developmental neurotoxicity study observed after a single oral dose were most appropriate for this risk assessment. This is also the dose at which effects were observed in offspring; therefore, use of the maternal NOAEL is protective for infants and children. Additionally, use of the LOAEL from the acute neurotoxicity study with a 3x uncertainty factor would result in a calculated NOAEL of 3 mg/kg/day and an acute RfD of 0.03 mg/kg. The HIARC determined that it was more conservative and protective of all populations (including females 13-50) to use the developmental neurotoxicity study.

2. Chronic Reference Dose (cRfD)

Study Selected: Chronic Toxicity - Dog **§** 83-1, OPPTS 870.4100

MRID Nos.: 40166701, 42022801

Executive Summary: In a chronic toxicity study (MRID No. 40166701), carbaryl (99%) was administered in the diet to 6 beagle dogs/sex/group at doses of 0, 125, 400 or 1250 ppm for one year. Nominal doses were 3.1, 10 and 31.3 mg/kg/day.

There were no deaths during the study. With the 1250 ppm females, there was an increased incidence of clinical signs of toxicity, including emesis, lacrimation, salivation and tremors. Mean body weight gain was decreased (50%) in the 1250 ppm females for weeks 0-6. Mean food consumption was decreased (16-24%, not statistically significant) in the 1250 ppm females at multiple time periods during the study. No treatment-related ophthalmoscopic changes were observed. There was a statistically significant increase in white blood cell and segmented neutrophil counts at some of the testing intervals for the 1250 ppm group males. Albumin levels were significantly decreased (9-11%) at all of the testing periods in the 1250 ppm females. Plasma cholinesterase (ChE) levels in males were significantly decreased in the 400 ppm (30-36%↓) and 1250 ppm (58-66%↓) groups at all testing intervals (weeks 5, 13, 26 and 52). Plasma ChE levels in females were significantly decreased at most intervals in the 125 ppm group (12-23%↓), 400 ppm group (9-31%↓) and 1250 ppm group (47-60↓). RBC ChE levels in males were significantly decreased in the 400 ppm group (23-28%↓at weeks 5 and 13) and 1250 ppm group (46-56%↓ for all intervals). RBC ChE levels in females were significantly decreased in the 400 ppm group (29-34% ↓at weeks 5, 13 and 26) and 1250 ppm (29-38% ↓for all intervals). Brain ChE in males was not statistically significantly decreased but biologically decreased in the 400 ppm group (32%↓) and 1250 ppm group (25%↓). Brain ChE in females was significantly decreased (20-36% ↓) in all the groups. No treatment-related effects were seen in urinalysis parameters.

At necropsy, there was a statistically significant increase in the absolute weight of the liver/gall bladder in the 1250 ppm group males. Relative and liver-to-brain weights were also increased but not significantly. There was a dose-related decrease in the absolute, relative and organ-to-brain weights of the pituitary in males, although none of the changes was statistically significant. There was also a significant decrease in the relative weight of the thyroid in this group. However, since there were no accompanying microscopic changes in these organs, the toxicological significance of these organ weight effects is questionable.

The LOAEL for systemic toxicity was 1250 ppm (31.3 mg/kg/day) based on an increased incidence of clinical signs (females), decreased body weight and food consumption (females) and alterations in clinical pathology parameters (both sexes); NOAEL was 400 ppm (10 mg/kg/day).

The LOAEL for plasma cholinesterase inhibition was 125 ppm (3.1 mg/kg/day) for females; a NOAEL was not established. The LOAEL for plasma cholinesterase inhibition was 400 ppm (10 mg/kg/day) for males; the NOAEL was 125 ppm (3.1 mg/kg/day).

The LOAEL for RBC cholinesterase inhibition was 400 ppm (10 mg/kg/day) for males and females; the NOAEL was 125 ppm (3.1 mg/kg/day).

The LOAEL for brain cholinesterase inhibition was 125 ppm (3.1 mg/kg/day) for females; a

NOAEL was not established. The LOAEL for brain cholinesterase inhibition was 400 ppm (10 mg/kg/day) for males; the NOAEL was 125 ppm (3.1 mg/kg/day).

In a five-week study (MRID # 42022801) done to upgrade the chronic study, carbaryl (99.3% a.i.) was administered in the diet to six beagles/sex/group at doses of 0, 20, 45 or 125 ppm. Actual mg/kg/day doses for males were 0, 0.59, 1.43 and 3.83 mg/kg/day, respectively; doses for females were 0, 0.64, 1.54 and 4.11 mg/kg/day, respectively. The following parameters were measured: clinical observations, body weights, food consumption, ophthalmoscopic examinations, plasma and RBC cholinesterase (at days -11, -8 and -5 pretest and then days 14 and 32 of the study), brain cholinesterase (at termination) and gross necropsies. This study was conducted to complete the information needed to satisfy the chronic toxicity study requirement in nonrodent species.

There were no deaths or treatment-related clinical signs of toxicity. There were no treatment-related effects on body weights, food consumption or ophthalmoscopic examinations. In males, there was a statistically and biologically significant decrease in plasma cholinesterase for the 125 ppm (22%↓) group.

The LOAEL for systemic toxicity and for RBC and brain cholinesterase inhibition was >125 ppm (males: 3.83 mg/kg/day; females: 4.11 mg/kg/day); the NOAEL was ≥ 125 ppm.

The LOAEL for plasma cholinesterase inhibition for males was 125 ppm; the NOAEL was 45 ppm (1.43 mg/kg/day). The LOAEL for cholinesterase inhibition for females was >125 ppm; the NOAEL was ≥ 125 ppm.

Dose and Endpoint for Establishing RfD: LOAEL =3.1 mg/kg/day based on plasma and brain cholinesterase inhibition in females.

Uncertainty Factor(s): 300 [10 for intra species variation, 10 for interspecies variation and 3 for use of a LOAEL].

$$\textbf{Chronic RfD} = \frac{\text{3.1 mg/kg/day (LOAEL)}}{\text{300 (UF)}} = \textbf{.01 mg/kg/day}$$

Comments about Study/Endpoint/Uncertainty Factor: The HIARC established the Chronic RfD using the LOAEL of 3.1 mg/kg/day in the chronic toxicity study in dogs. Since a NOAEL was not established in this study, an additional uncertainty factor of 3X was applied to the LOAEL (i.e, UF_L). The HIARC determined that 3X is adequate to account for the lack of a NOAEL in this case because: 1) the study was well-conducted and there are sufficient data from subchronic and other chronic studies in other species that support cholinesterase inhibition as the critical effect for Carbaryl; 2) the data indicate that the dog is more sensitive to the cholinergic effects of Carbaryl and using this species to establish the RfD provides additional protection for the effects seen in the rat (including the reproduction and developmental neurotoxicity studies); 3) the magnitude of plasma cholinesterase inhibition (12-23% decrease)

seen in this study was comparable to the magnitude of inhibition (22%) seen in the 5-week study in dogs - indicating no cumulative effect following long-term exposure; 4) The cholinesterase inhibition seen in females at the LOAEL in this study was not accompanied by clinical signs (response was not judged to be severe); and 5) no inhibition was seen for any cholinesterase compartment in males at this dose (response was seen in only one sex).

3. Incidental Oral Exposure: Short-Term (1 - 30 days)

Study Selected: Developmental Neurotoxicity Study in Rats §81-8; OPPTS 870.6300

MRID Nos.: 44393701, 45456701, 45456702, 45456703

Executive Summary: See Acute Reference Dose (aRfD) - General Population

Dose and Endpoint for Risk Assessment: Maternal NOAEL of 1 mg/kg based on alterations in FOB parameters; decreases in RBC, whole blood, plasma and brain cholinesterase

Comments about Study/Endpoint: The study route and duration are appropriate for this risk assessment. The effects (FOB alterations) were observed after a single dose and continued after multiple days of dosing and are appropriate for the population of concern (infants and children). Decreases in RBC, whole blood, plasma and brain cholinesterase were observed at Week 4 of dosing.

4. Incidental Oral Exposure: Intermediate-Term (1 - 6 Months)

Study Selected: Subchronic Neurotoxicity Study Study Guideline#: § 81-8, OPPTS 870.6200

MRID No.: 44122601

Executive Summary: In a subchronic neurotoxicity study, 12 Crl:CD(SD)BR rats/sex/group were administered technical carbaryl (99.1%) by gavage at doses of 0, 1, 10 or 30 mg/kg/day for 13 weeks. Cholinesterase (RBC, whole blood, plasma and brain) determinations were done on an additional three groups of five rats/sex/group at Weeks 4, 8 and 13. Neurobehavioral screening, consisting of Functional Observational Battery (FOB) and motor activity evaluations, was performed prior to treatment and during Weeks 4, 8 and 13. At terminal sacrifice, six animals/sex/dose were anesthetized and perfusion fixed *in situ* for neuropathological evaluation.

There were no deaths during the study. There was an increased incidence of clinical signs of toxicity, including slight and moderate salivation and tremors, in the 30 mg/kg/day males and females. Body weight over the course of the study was statistically significantly decreased in the 30 mg/kg/day males (14%) and females (15%). Body weight gain for these groups was decreased 27% in males and 37% in females, compared to controls. Food consumption was decreased during most of the study for the 30 mg/kg/day males and females. Males and females in the 30 mg/kg/day group had a statistically significant decrease in RBC (M:42-46%;

F:52-55%), whole blood (M: 49-51%; F: 59-63%) and plasma (M: 63-69%; F: 63-69%) at most of the testing periods. Males and females in the 10 mg/kg/day group had a statistically significant decrease in RBC (M: 26-38%; F: 17-24%); whole blood (M: 30-41%; F: 21-26%) and plasma (M:43-48%; F: 23-30%). There was a statistically significant decrease in brain cholinesterase in males and females in the 10 mg/kg/day (M: 27-61%; F: 20-58%) and 30 mg/kg/day (M: 36-80%; F: 50-73%) groups. For the 1 mg/kg/day males, there were statistically significant decreases in whole blood (13%) at week 13 and for plasma (20%) at week 8. These changes are not considered toxicologically significant since they occurred infrequently and were relatively minor effects.

Multiple qualitative and quantitative FOB parameters were affected in the 10 and 30 mg/kg/day males and females, including the following: slight tremors, gait alterations, pinpoint pupils, increased salivation, reduced extensor thrust, decreased pinna reflex, reduced number of rearings, decreased vocalizations, decreased body temperature and decreased forelimb grip. Reduced number of defecations was observed only at 30 mg/kg/day. There was an occasional alteration at the 1 mg/kg/day dose. At week 8, males had a very slight increase in the incidence of pinpoint pupils (incidence in control, 1, 10 and 30 mg/kg/day groups was 0/12, 1/12, 6/12 and 10/12, respectively). A statistically significant decrease in forelimb grip was observed at week 4 in males (values for control, 1, 10 and 30 mg/kg/day groups were 1060.8, 943.8, 943.8 and 950.0, respectively). The number of defecations was statistically reduced in females at week 13 (mean number of defecations in control, 1, 10 and 30 mg/kg/day groups were 1.4, 0.2, 0.5 and 0.0, respectively). The toxicological significance of these effects is questionable since the incidence was either low or there was no dose-response relationship.

Motor activity was statistically significantly decreased in the 30 mg/kg/day males at Week 4 and the 30 mg/kg/day females at Weeks 4 and 8.

On necropsy, there was an increased incidence of dark areas in the meninges of the 30 mg/kg/day males; these animals had an increased incidence of hemorrhage on microscopic examination. One female in the 30 mg/kg/day group also had retinal atrophy. There were no differences in brain length or width measurements.

The LOAEL for neurotoxicity was 10.0 mg/kg/day based on an increased incidence of FOB changes; the NOAEL was 1.0 mg/kg/day. The LOAEL for cholinesterase inhibition was 10.0 mg/kg/day based on statistically significant decreases in RBC, whole blood, plasma and brain cholinesterase; the NOAEL was 1.0 mg/kg/day.

Dose and Endpoint for Risk Assessment: NOAEL = 1.0 mg/kg/day based on plasma, whole blood, RBC and brain cholinesterase inhibition and FOB changes at 10 mg/kg/day.

Comments about Study/Endpoint: The study was selected because the route of administration (oral) and the duration (90 days) are appropriate for this risk assessment. It is supported by the five-week dietary study in dogs (MRID 4202801) done to upgrade the chronic toxicity study in which the NOAEL in males was 1.43 mg/kg/day based on plasma cholinesterase inhibition at 3.83 mg/kg/day. This dose and endpoint are appropriate for the population of concern (infants

and children).

5. Dermal Absorption

Dermal Absorption Factor: A dermal absorption factor of 12.7% was selected at the July 7, 1998 HIARC meeting. The value was derived from a dermal absorption study (MRID # 43552901) with a 43.9% formulation using the 10-hour absorption rate.

6. Short-Term Dermal (1 - 30 days) Exposure

Study Selected: 4-Week Dermal Study in Rats § Number: non-guideline

MRID No.: 45630601

Executive Summary: In a non-guideline four-week dermal toxicity study (MRID 45630601), Carbaryl Technical (99.49% a.i., Lot 211048078) was applied to the shaved skin of 10 Crl: CD (SD)IGS BR rats/sex/dose at dose levels of 0, 20, 50 or 100 mg/kg bw/day, 6 hours/day for 5 days/week during a 4-week period. The parameters measured included the following: clinical observations, body weight, body weight gain, food consumption, RBC and brain cholinesterase and signs of dermal irritation.

There was no treatment-related effect on mortality, clinical observations, body weight or dermal irritation. The only statistically significant body weight gain changes were a decrease (27%) in the 100 mg/kg/day males during Days 5 to 12 and an increase (37%) in 50 mg/kg/day males during Days 19 to 26. However, there were non-significant decreases in the 100 mg/kg/day males at Days -3 to 5 (16%), 12 to 19 (17%) and -3 to 26 (12%) which are considered toxicologically significant.

The only statistically significant decreases in food consumption were in the 50 mg/kg/day females on Days 12 to 19 and 50 and in the 100 mg/kg/day females on Days 19 to 26. The effects are not considered treatment-related as there was no dose-response and the decreases were minimal (9% and 8% in the 50 and 100 mg/kg/day groups, respectively).

RBC cholinesterase was measured before dosing on Day -4 and on Days 1, 8, 15 and 22. The only statistically significant effects were in the 100 mg/kg/day males at Days 8 (11% decrease) and 22 (13%). Using the repeated measures statistical test, there were also significant decreases in the 50 and 100 mg/kg/day females (11% and 10%, respectively) on Day 22. These effects were determined to be not toxicologically significant because they were inconsistent.

Measurements were also performed within 1 hour after test material removal on Days 5, 12, 19, and 26. Statistically significant decreases were observed in the 50 mg/kg/day (12% decrease) and 100 mg/kg/day (15%) males on Day 5 and in the 100 mg/kg/day males on Days 12 (21%) and 19 (16%). Using the repeated measures statistical test, there was also a significant decrease (10%) in the 50 mg/kg/day males on Day 12. In females, statistically significant decreases were observed in the 50 and 100 mg/kg/day groups on Days 5 (13% and 12%, respectively) and Day 12 (20% and 13%, respectively).

Brain cholinesterase was statistically significantly decreased in the 50 mg/kg/day males (15%) and in the 100 mg/kg/day males (15%) and females (24%). There was also a non-significant decrease in the 50 mg/kg/day females (9%).

The systemic LOAEL is conservatively established at 50 mg/kg/day based on statistically significant decreases in RBC cholinesterase in males and females and brain cholinesterase in males. The systemic NOAEL is 20 mg/kg/day.

The dermal LOAEL was not established. The dermal NOAEL was 100 mg/kg/day.

This 4-week dermal toxicity study in the rat is **acceptable (non-guideline)**. The study was intended to establish endpoints for short-term and intermediate-term occupational and residential postapplication dermal exposure. Although the study does not meet guideline requirements, it is useful for risk assessment for the following reasons: 1) in all oral studies in which cholinesterase was measured, it was the most sensitive endpoint; therefore, other guideline parameters would most likely not establish a lower LOAEL; 2) plasma cholinesterase was not measured; however, in all the oral studies in rats, all three compartments (plasma, RBC and brain) were affected at the same dose level. Therefore, it is likely that plasma cholinesterase would not have been inhibited at a lower level, especially given the minimal effects on RBC and brain cholinesterase.

Dose and Endpoint for Risk Assessment: 20 mg/kg/day (NOAEL) based on significant decreases in RBC and brain cholinesterase in males and females and brain cholinesterase in males at 50 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The study route and duration are appropriate for this risk assessment. Although the study was not conducted according to the 21/28-day dermal toxicity guidelines (OPPTS 870.3200), the most sensitive parameter (cholinesterase) was measured. The NOAEL (20 mg/kg/day) is protective for offspring effects observed in the developmental neurotoxicity study for the following reasons: 1) the NOAEL/LOAEL in the dermal toxicity study was conservatively established at 20/50 mg/kg/day; the decreases in RBC and brain cholinesterase were minimal at 50 mg/kg/day; 2) The dermal absorption factor is conservative in that the study was conducted with a formulation which includes inert ingredients that may enhance absorption. In addition, the highest percent of absorption is traditionally used; 3) In the developmental neurotoxicity study, the oral NOAEL and LOAEL were 1 and 10 mg/kg/day, respectively. Using the dermal absorption factor of 12.7%, the dermal equivalents are 8 and 80 mg/kg/day, respectively; 4) the morphometric alterations seen in the pups in the developmental neurotoxicity study were minimal at 10 mg/kg/day (equivalent dermal dose: 80 mg/kg/day) and were seen in the presence of cholinesterase inhibition (plasma, RBC and brain) in the maternal animals; 5) The 50 mg/kg/day LOAEL in the dermal toxicity study is lower than the equivalent dermal dose of 80 mg/kg/day and thus would be protective of the concerns for the morphometric alterations in the developmental neurotoxicity study.

7. Intermediate-Term Dermal (1 - 6 Months) Exposure

Study Selected: 4-Week Dermal Study in Rats § Number: non-guideline

MRID No.: 45630601

Executive Summary: See Short-term Dermal Exposure

Dose/Endpoint for Risk Assessment: 20 mg/kg/day (NOAEL) based on significant decreases in RBC and brain cholinesterase in males and females and brain cholinesterase in males at 50 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The route is appropriate for this risk assessment. Although the duration of the study was at the lower end of the human exposure range, there were significant decreases in brain cholinesterase at the end of the study, indicating that no tolerance of the chemical occurred earlier in the study. In addition, there was no evidence from the RBC cholinesterase data that inhibition worsened with increasing duration. Therefore, a lower NOAEL would not be expected if the study duration were longer.

8. Long-Term Dermal (> 6 Months) Exposure

Study Selected: Chronic Toxicity - Dog **§** 83-1, OPPTS 870.4100

MRID Nos.: 40166701, 42022801

Executive Summary: See Chronic Reference Dose (cRfD)

Dose and Endpoint for Risk Assessment: LOAEL =3.1 mg/kg/day based on plasma and brain cholinesterase inhibition in females.

Comments about Study/Endpoint: No long-term dermal studies are available. The duration of exposure in this oral study is appropriate. A dermal absorption factor of 12.7% should be applied. The reasons for selecting this oral study to assess long-term exposure are described under Chronic Reference Dose (cRfD).

9. Short-Term Inhalation (1-7 days) Exposure

Study Selected: Developmental Neurotoxicity Study **§**81-8; OPPTS 870.6300

MRID No.: 44393701, 45456701, 45456702, 45456703

Executive Summary: See 2.1 Acute Reference Dose (aRfD) - General Population

Dose and Endpoint for Risk Assessment: Maternal NOAEL of 1 mg/kg/day based on alterations in FOB parameters on the first day of dosing at 10 mg/kg/day (LOAEL)

Comments about Study/Endpoint: No inhalation toxicity studies are available. The HIARC determined that the study is appropriate for the short-term exposure time period because effects (FOB alterations) were observed after a single dose and continued after multiple days of dosing. Absorption via the inhalation route is presumed to be equivalent to oral absorption.

10. Intermediate-Term Inhalation (7 Days to Several Months) Exposure

Study Selected: Subchronic Neurotoxicity Study § 81-8, OPPTS 870.6200

MRID Nos.: 44122601

Executive Summary: See 2.3.2 Intermediate-Term Incidental Oral Exposure

Dose/Endpoint for Risk Assessment: NOAEL = 1.0 mg/kg/day based on plasma, whole blood, RBC and brain cholinesterase inhibition and FOB changes at 10 mg/kg/day.

Comments about Study/Endpoint: No inhalation studies are available. Absorption via the inhalation route is presumed to be equivalent to oral absorption.

11. Long-Term Inhalation (Longer than 6 months) Exposure

Study Selected: Chronic Toxicity - Dog **§** 83-1, OPPTS 870.4100

MRID Nos.: 40166701, 42022801

Executive Summary: See Chronic Dietary (cRfD)

Dose and Endpoint for Risk Assessment: LOAEL of 3.1 mg/kg/day based on plasma and brain cholinesterase inhibition in females in the 1 year study.

Comments about Study/Endpoint: No inhalation studies are available. The reasons for selecting this oral study to assess long-term exposure are described under 2.2 Chronic Reference Dose (cRfD). Absorption via the inhalation route is presumed to be equivalent to oral absorption.

12. Margins of Exposure

The target Margins of Exposure (MOEs) for **occupational** exposure risk assessments are as follows:

Route / **Duration**	**Short-Term (1-30 Days)**	**Intermediate-Term (1 - 6 Months)**	**Long-Term (> 6 Months)**
Dermal	100	100	300
Inhalation	100	100	300

The MOEs for short- and intermediate-dermal and inhalation exposures may be combined for occupational exposure risk assessment because the toxicity endpoints (ChEI) and the MOEs

for these routes of exposure are the same.

The target MOEs for **residential** exposure risk assessments will be determined by the FQPA Safety Factor Committee.

13. Recommendation for Aggregate Exposure Risk Assessments

As per FQPA, 1996, when there are potential residential exposures to the pesticide, aggregate risk assessment must consider exposures from three major sources: oral, dermal and inhalation exposures. A common endpoint of concern (cholinesterase inhibition) was identified for short-, intermediate- and long-term oral, dermal and inhalation (oral equivalent) exposures. Therefore, these routes can be aggregated for these scenarios for the appropriate populations.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID No.:42918801

Discussion of Tumor Data:. Male rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls, for thyroid follicular cell adenomas and combined adenomas and/or carcinomas, and urinary bladder transitional cell papillomas, carcinomas, and combined papillomas and/or carcinomas, all at $p < 0.01$.

Female rats had significant increasing trends in urinary bladder transitional cell papillomas, carcinomas, and combined papillomas and/or carcinomas, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls for urinary bladder transitional cell papillomas ($p < 0.05$), carcinomas ($p < 0.05$), and combined carcinomas and/or papillomas ($p < 0.01$).

Adequacy of the Dose Levels Tested: At meetings on October 27 and December 8, 1993, the HED Cancer Peer Review Committee determined that the 7500 ppm dose was excessive based on the following findings: 1) changes in body weight gain during week 13 for males and females by 40% and 52%, respectively, as compared to controls; 2) decreased food efficiency; 3) alterations in hematology and clinical chemistry; and 4) decreases in plasma, RBC and brain cholinesterase at weeks 53 and 105. The CPRC also concluded that the mid dose (1500 ppm) was not adequate for carcinogenicity testing. The November 7, 2001 CARC meeting affirmed that the high dose was excessive and the mid dose was not sufficiently high enough to test the carcinogenic potential of carbaryl in rats.

2. Carcinogenicity Study in Mice

MRID No.: 42786901

Discussion of Tumor Data: Male mice had significant increasing trends in kidney tubule cell

adenomas ($p < 0.05$), carcinomas ($p < 0.05$) and combined adenomas and/or carcinomas ($p < 0.01$). There was also a significant difference in the pair-wise comparison of the 8000 ppm dose group with the controls for combined kidney tubule cell adenomas and/or carcinomas at $p < 0.05$. There were significant differences in the pair-wise comparisons of all dose groups (100, 1000 and 8000 ppm) with the controls for hemangiosarcomas, all at $p < 0.05$. There were significant differences in the pair-wise comparisons of 1000 and 8000 ppm dose groups with the controls for hemangiomas and/or hemangiosarcomas combined, both at $p < 0.05$.

Female mice had significant increasing trends in hepatocellular adenomas and adenomas, carcinomas and/or hepatoblastomas combined, both at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls for hepatocellular adenomas at $p < 0.05$ and for hepatocellular adenomas, carcinomas and/or hepatoblastomas combined at $p < 0.01$. There was a significant increasing trend at $p < 0.01$, and a significant difference in the pair-wise comparison of the 8000 ppm dose group with the controls at $p < 0.05$, for hemangiosarcomas. There was also a significant increasing trend for hemangiomas and/or hemangiosarcomas combined at $p < 0.05$.

Adequacy of the Dose Levels Tested: At meetings on October 27 and December 8, 1993, the HED Cancer Peer Review Committee concluded that the 8000 ppm dose was excessive based on the significantly decreased body weight gain in males (33%) and females (19%) during week 13, a significant decrease in RBC and brain cholinesterase activity, clinical signs of toxicity and histopathological changes in the bladder, kidneys and spleen in both sexes. The November 7, 2001 CARC meeting affirmed that the high dose was excessive.

3. Carcinogenicity and Other Studies in p53 Knockout Mice

In a special, non-guideline study (MRID 45281801), heterozygous p53-deficient (knockout) male mice (20/group) were administered carbaryl in the diet at concentrations of 0, 10, 30, 100, 300, 1000 and 4000 ppm (approximately 0, 1.8, 5.2, 17.5, 51.2, 164.5 and 716.6 mg/kg/day, respectively) for six months. The doses selected for this study were based on two 28-day studies (MRID 45236603) in wild-type mice in which body weight decreases were observed at 4000 and 8000 ppm concentrations of carbaryl in the diet. A validation study (MRID 45281802) demonstrated that vascular tumors occur in heterozygous p53-deficient mice within 6 months of administration of a known genotoxic carcinogen (urethane). These studies were conducted to demonstrate that carbaryl is a non-genotoxic carcinogen. In the standard mouse carcinogenicity study (MRID 42786901) at dietary concentrations of 0, 100, 1000 or 8000 ppm, there was an increased incidence of vascular neoplasms (hemangiomas and hemangiosarcomas) in all treated males and in the 8000 ppm group females. There was an increased incidence of adenomas, multiple adenomas and carcinomas of the kidney in the 8000 ppm group males. The incidence of hepatic neoplasms (adenomas, carcinomas and one hepatoblastoma) was increased in the 8000 ppm group females. At meetings on October 27 and December 8, 1993, the HED Cancer Peer Review Committee concluded that the 8000 ppm dose was excessive. Therefore, the relevance of tumors at this dose was questionable.

In the p53 knockout mouse study with carbaryl, there was a slight decrease in body weight and

food consumption in the 4000 ppm group. No other treatment-related effects were observed, except globular deposits in the urinary bladder were observed in a high proportion of the mice treated at 100 ppm of carbaryl and above with a dose-related increase in incidence and severity. There was no evidence of local irritation or hypertrophy of the bladder epithelium. There was no evidence of neoplastic or preneoplastic changes in the vascular tissue of any organs examined.

The study is classified **Acceptable (non-guideline)**. This is a special study not submitted to fulfill a data requirement.

4. Classification of Carcinogenic Potential.

The carcinogenic potential of carbaryl was evaluated by the HED Carcinogenicity Peer Review Committee on October 27 and December 8, 1993 (May 12, 1994 report). The Committee concluded that carbaryl induced tumors at multiple sites in the rat and mouse at doses considered to be excessively toxic. Only hemangiosarcomas in the CD-1 male mouse occurred at a dose which was considered adequate and not excessive. The Committee concluded that carbaryl should be classified as a Group C - possible human carcinogen. Both the low-dose extrapolation (Q_1*) approach and a margin of exposure (MOE) approach were suggested as methods of quantifying the cancer risk in humans. In addition, a RfD approach was suggested to provide the most sensitive non-cancer health endpoint for comparison to the linear and MOE approaches. The Committee requested additional metabolism studies and genotoxicity studies to: 1) direct the selection of the more appropriate quantitative approach; and 2) provide insight into the significance of tumors seen only at excessively toxic doses.

Additional metabolism studies were submitted and evaluated by a subgroup of the HED Cancer Assessment Review Committee (CARC) in a memorandum signed October 5, 1998. The subgroup concluded that the available metabolism studies were not adequate to support a nonlinear mode of action and recommended that the default linear approach be used for risk quantitation.

In 1996, the registrant convened a Pathology Working Group (PWG) which reevaluated all histopathology findings of both the two-year rat and mouse studies. The results of this PWG are discussed below with the study summaries.

At a November 7, 2001 meeting, the HED CARC classified carbaryl as **"Likely to be carcinogenic to humans"** based on a statistically significant increase in hemangiosarcomas in male mice at all doses tested (100, 1000 and 8000 ppm), all at $p<0.05$. In addition, there were preneoplastic lesions in the bladders of male rats at the mid dose (1500 ppm) which was not considered adequate for carcinogenicity testing. Bladder tumors were observed in male rats at the high dose which was considered excessive.

The unit risk, Q_1^* $(mg/kg/day)^{-1}$, of Carbaryl is 8.75×10^{-4} in human equivalents based on the 1996 PWG re-read of the male mouse hemangiosarcoma tumor rates.

IV. MUTAGENICITY

During the meetings on October 27, and December 8, 1993, the CPRC (1994) recommended that an *in vivo* cytogenetic assay in rodents be conducted to provide insight into the structural and/or numerical aberrations, which were observed in the gene mutation assay and reported in the open literature. In response to CPRC's request, a mouse micronucleus assay (MRID 44069301) was submitted to fulfill the guideline requirement but it was classified as unacceptable.

A recent review of the data from the submitted studies and the published literature were in general agreement and show that carbaryl is clastogenic *in vitro.* The wide variety of induced aberrations (both simple and complex) was consistent between the submitted study and the open literature. However, there are inconsistencies relative to the requirement for S9 activation.

Nevertheless, the two *in vivo* studies for micronuclei induction or chromosome aberrations were negative. Similarly, the 6-month p53 knockout transgenic mouse bioassay was negative up to a high level (4000 ppm, ≈720 mg/kg/day) that approached the limit dose for a mouse carcinogenicity assay. Carbaryl was also negative for DNA binding in the livers of mice treated with 8000 ppm for 2 weeks but the study was considered to be of limited sensitivity by the CARC Metabolism Subgroup (HED Document No. 012892). The same Subgroup identified epoxide intermediates of carbaryl which were found to be conjugated to glucuronide, "rapidly metabolized and excreted as any endogenous epoxide would be".

Overall, these findings indicate that carbaryl produces epoxides and its DNA reactivity is manifested as chromosomal aberrations in cultured mammalian cells. Other *in vitro* studies indicate carbaryl's effects on karyokinesis and cytokinesis, as well as stress genes associated with oxidative damage. Based on these considerations, it was concluded that there is a concern for mutagenicity, which is somewhat lessened because of the lack of an effect in *in vivo* mutagenicity studies.

GENE MUTATIONS

Mutagenicity - Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay (Ames test)

In a Salmonella/mammalian activation gene mutation assay (MRID 41370303), carbaryl technical (99.3%) was initially evaluated in the Salmonella typhimurium/microsome mutagenicity assay over a concentration range of 5 to 1000 µg/plate. The test material was not mutagenic, however the highest assayed dose was cytotoxic in S. typhimurium strains TA98 and TA100, but not in strains TA1535, TA1537, or TA1538. Accordingly, the assay was repeated with six concentrations (10 to 2000 µg/plate +/-S9). Results from the repeat assay indicated that 2000 µg/plate +/-S9 was cytotoxic in strains TA98 and TA100, and the remaining doses were not mutagenic. It is concluded, therefore, that carbaryl technical was assayed to an appropriately high concentration with no evidence of mutagenicity in a well-

conducted study. The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements (§84-2) of bacterial reverse mutation test.

Mutagenicity - Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster Ovary (CHO) Cells

In a mammalian cells in culture gene mutation assay in Chinese Hamster Ovary (CHO) Cells (MRIDs 41370302, 41420201), carbaryl technical (99.3%) was evaluated in two nonactivated and three S-9 activated Chinese hamster ovary (CHO) cell forward mutation assays. The findings from both nonactivated assays were in good agreement and indicated that over a concentration range of 1 to 300 µg/mL, the test material did not induce a mutagenic response. Doses ≥200 µg/mL were severely cytotoxic (<10% cell survival), and <50% of the cells survived exposure to ≥50 µg/mL. Carbaryl was less cytotoxic in the presence of S9 activation as indicated by increased survival at comparable levels in the preliminary cytotoxicity test (e.g., 29.5% survival at 62.5 µg/mL -S9 as compared with 95.7% survival at 62.5 µg/mL +S9) and the initial mutation assay (e.g., 18.1% survival at 100 µg/mL -S9 as compared with 46.8% at 100 µg/mL +S9). There was no definitive evidence of increased mutation frequencies (MFs) in this trial. The second S9-activated trial was aborted because of excessive cytotoxicity at test material levels of ≥10 µg/mL. Results from the third S9-activated trial (dose range: 1 to 80 µg/mL) showed severe cytotoxic effects at levels ≥60 µg/mL; no evidence of mutagenic effect was seen at the remaining doses.

The results of the assays provide no clear indication of a mutagenic response, however, the study does not fully support a negative conclusion. The conflicting cytotoxicity data for the S9-activated assays provide no assurance that the final S9-activated mutation assay was conducted over an appropriate dose range. The study is classified as **unacceptable/guideline** and **does not satisfy** the guideline requirements (§84-2) for an *in vitro* mammalian cell gene mutation test.

CHROMOSOME ABERRATIONS

Mutagenicity - Mammalian Cells in Culture Cytogenetic Assay

Carbaryl (technical) was assayed for clastogenic effects in both the presence and absence of S9 activation using Chinese hamster ovary (CHO) cells (MRID 41370301). Because of severe cell cycle delay, which was more pronounced without S9 activation, a 20-hour cell harvest was selected to evaluate seven nonactivated doses ranging from 5 to 100 µg/mL. In the presence of S9 activation, cells exposed to carbaryl at doses of 25, 50, 75, 100, 150, 200, 250, and 300 µg/mL were harvested 30 hours post treatment. Results indicated that the nonactivated test material was more cytotoxic than the S9-activated test material (*i.e.*, few metaphases were recovered at 75 and 100 µg/mL , and moderate to slight cytotoxic effects were seen at doses ≥10.0 µg/mL). With the exception of a single rare complex aberration (quadriradial) scored at the 50.0-µg/mL dose level, there was no evidence of a clastogenic effect. By contrast, in the S9-activated assays, all scored doses (150, 200, 250, and 300 µg/mL) at both harvest times induced significant ($p \leq 0.01$) increases in the percentage of cells with aberrations. The

majority of S9-activated doses (both harvests) also induced significant ($p \leq 0.01$) increases in the percentage of cells with >1 aberration. At both the 20- and 30-hour harvest times, cytotoxicity (*i.e.*, reduced monolayers, dead cells, and/or reduced mitotic cells) were observed at levels ≥200 µg/mL. Induced structural damage included simple (*i.e.*, chromatid and chromosome breaks) and complex aberrations (*i.e.*, triadials, quadriradials, complex rearrangements, dicentrics and rings). The data show little or no dose responsiveness and the lowest reactive level of carbaryl was not determined. It was concluded, however, that the study was technically sound and, therefore, **acceptable/guideline**. The study **satisfies** the Guideline requirements (§84-2) for an *in vitro* mammalian cell chromosomal aberration test.

Mutagenicity - Mouse Micronucleus Test

In a mouse micronucleus assay (MRID No: 44069301), groups of five male and five female CD-1 mice received single oral gavage administrations of 50, 100 or 200 mg/kg carbaryl (99.9%) once daily for 2 days. Based on analytical determinations, average daily doses were ≈34, 79 or 180 mg/kg. Mice were sacrificed at 24 and 48 hours postadministration of the second dose and harvested bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs). The test material was delivered as suspensions prepared in 0.5% carboxymethyl cellulose.

The minimal toxicity (i.e., lethargy which lasted for 2 hours) in the absence of cytotoxicity to the target cells does not support the testing of the maximum tolerated dose (MTD). The positive control induced the expected high yield of MPEs in males and females. Carbaryl did not induce a clastogenic or aneugenic effect in either sex at any dose or sacrifice time. However, there was no convincing evidence that the MTD was achieved**.** The study is classified as **unacceptable/guideline** and **does not satisfy** the guideline requirements(§84-2; OPPTS 870.5385) for *in vivo* cytogenetic mutagenicity data.

OTHER MUTAGENIC EFFECTS

Mutagenicity - UDS Assay

In a UDS Assay in primary rat hepatocytes (MRID 41370301), under the conditions of two independent trials, six doses of carbaryl technical (99.3%) ranging from 0.5 to 25.0 µg/mL in the first assay and six doses ranging from 5.0 to 25.0 µg/mL in the repeat assay did not induce an appreciable increase in the net nuclear grain counts of treated rat hepatocytes. Doses >25.0 µg/mL were severely cytotoxic; reduced cell survival (≈25%) was observed at 25.0 µg/mL in both assays. Although an increase in the percentage of cells with ≥ 6 grains per nucleus was seen in the initial test, the increase was confined to a single dose (10 µg/mL) and was not dose-related or reproducible. The study demonstrated that carbaryl is not genotoxic in this test system at doses of 5.0 to 25.0 µg/mL. The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements (§84-2) for a unscheduled DNA synthesis in mammalian cells in culture.

STUDIES FROM THE OPEN LITERATURE

Studies in the open literature indicate that Carbaryl is not mutagenic in bacteria but produced conflicting results in Chinese hamster V79 gene mutation assays [negative in the study of

Onfelt and Klasterska[6] but weakly positive minus S9 metabolic activation as reported by Ahmed et al.[7] Nonactivated carbaryl induced aneuploidy and sister chromatid exchanges in V79 cells; the addition of S9 or an excess of glutathione eliminated these responses (Onfelt and Klasterska [8,2]). In the former study, multiple chromatid exchanges (quadriradials and complex rearrangements) plus chromosome breaks were also induced by 100 mM carbaryl; this effect was largely abolished by the simultaneous addition of S9 or glutathione. There are positive data for DNA damage in a human lymphoblastoid cell line (induction of CYP1A1 genes); carbaryl also activated other stress genes known to be sensitive to oxidative damage (Delescluse *et al.*[9]). Also, carbaryl causes depolymerization of spindle microtubules and an apparent uncoupling of karyokinesis and cytokinesis in cultured V79 cells (Renglin *et al*,[10,11]).

In contrast to the *in vitro* data, carbaryl administered by oral gavage at 1/3 of the LD_{50} (146 mk/kg/day) for 2 consecutive days was negative for micronuclei induction in Swiss albino male mice (Usha Rani et al.[12]). Carbaryl was also negative for the induction of chromosome aberrations in bone marrow cells of Syrian hamsters treated with 1/10, 1/5 and ½ of the LD_{50} and the LD_{50} (Dzwonkowska and Hubner[13]).

A micronucleus study was required in previous HIARC reports as the study reviewed (MRID

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[6]Onfelt, A., Klasterska, I. (1984). Sister -chromatid exchanges and thioguanine resistance in V79 Chinese hamster cells after treatment with the aneuploidy-inducing agent carbaryl +/- S9 mix. Mutat Res 125(2): 269-274

[7]Ahmed, F.E., Lewis, N.J., Hart, R.W. (1977). Pesticide induced ouabain resistant mutants in Chinese hamsterV79 cells. Chem Biol Interact, 19:369-374.

[8] Onfelt, A., Klasterska, I. (1983). Spindle disturbances in mammalian cells II. Induction of viable aneuploidy/polyploidy cells and multiple chromatid exchanges after treatment of V79 Chinese hamster cells with carbaryl, modifying effect of glutathione and S9. Mutat Res 119: 319-330.

[9]Delescluse, C. *et al* (2001). Induction of cytochrome P450 1A1 gene expression, oxidative stress, and genotoxicity by carbaryl and thiabendazole in transfected human HepG2 and lymphoblastoid cells. Biochem Pharmacol.61(4):399-407.

[10]Renglin, A., Olsson A., Wachtmeister, C., Onfelt, A. (1998). Mitotic disturbance by carbaryl and the metabolite 1-naphthol may induce kinase-mediated phosphorylation of 1-naphthol to the protein phosphatase inhibitor 1-naphthyl phosphate. Mutagenesis 13: 345-352.

[11]Renglin, A., Harmala-Brasken, A., Eriksson, J., Onfelt, A. (1999). Mitotic aberrations by carbaryl reflect tyrosine kinase inhibition with coincident up-regulation of serine/threonine protein phosphatase activity: implications for coordination of karyokinesis and cytokinesis. Mutagenesis 14: 327-333.

[12]Usha Rani, M.V., Reddi, O.S. and Reddy, P.P. (1980). Mutagenicity Studies Involving Aldrin, Endosulfan, Dimethoate, Phosphamidon, Carbaryl and Ceresan. Bull Environm. Contam. Toxicol 25:277-282.

[13]Dzwonkowska, A., Hubner, H. (1986). Induction of chromosomal aberrations in the Syrian hamster by insecticides tested in vivo. Arch Toxicol 58(3):152-156.

44069301) was classified as unacceptable because a MTD was not reached. However, as discussed above, there were two studies from the open literature testing carbaryl up to the LD50 or 1/3 of the LD50, which were higher than the high dose in the micronucleus assay and negative. The HIARC considered these studies well-conducted and the results reliable. Therefore, the requirement for this study is satisfied.

III. DATA GAPS / REQUIREMENTS

90-day inhalation study with cholinesterase measurements

IV. ACUTE TOXICITY

Acute Toxicity of Carbaryl

Guideline No.	Study Type	MRIDs #	Results	Toxicity Category
81-1	Acute Oral - rat	00148500	LD_{50} for males = 302.6 mg/kg; for females = 311.5 mg/kg; combined = 301.0 mg/kg	II
81-2	Acute Dermal - rabbit	00148501	LD_{50} > 2000 mg/kg	III
81-3	Acute Inhalation - rat	00148502	LC_{50} > 3.4 mg/L	IV
81-4	Primary Eye Irritation	00148503	not a primary eye irritant	IV
81-5	Primary Skin Irritation	00148504	not a primary skin irritant	IV
81-6	Dermal Sensitization	00148505	negative	
81-7	Acute Delayed Neurotoxicity (Hen)	*	negative at 2000 mg/kg (approximate LD_{50})	
81-8	Acute Neurotoxicity - rat	43845201-43845204	systemic LOEL = 10 mg/kg for males and females based on significant inhibition of RBC, plasma, whole blood and brain cholinesterase; NOEL < 10 mg/kg	

* Carpenter, C.P., Weil, C.S., Palm, P.E., Woodside, N.W., Nair, J. H. and Smyth, H.F. Mammalian Toxicity of 1-napthyl-N-methyl carbamate (Sevin Insecticide). J. Agric. Food Chem. 9(1): 30-39, 1961.

VIII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

Summary of Toxicology Endpoint Selection for Carbaryl

Exposure Scenario	Dose (mg/kg/day) UF /MOE	Hazard Based Special FQPA Safety Factor	Endpoint for Risk Assessment
Dietary Risk Assessments			
Acute Dietary general population including infants and children	NOAEL = **1** UF = **100** **Acute RfD** = **0.01** mg/kg/day	1	**Developmental Neurotoxicity - rat** LOAEL = 10 mg/kg/day based on an increased incidence of FOB changes on the first day of dosing in maternal animals
Chronic Dietary all populations	LOAEL= **3.1** UF = **300** **Chronic RfD** = **0.01** mg/kg/day	1	**Chronic toxicity - dog** LOAEL = 3.1 mg/kg/day based on plasma and brain cholinesterase inhibition in females.
Incidental Oral Short-Term (1 - 30 Days) Residential Only	NOAEL= **1** **MOE=** TBD	1	**Developmental Neurotoxicity - rat** LOAEL = 10 mg/kg/day based on an increased incidence of FOB changes and decreases in RBC, whole blood, plasma and brain cholinesterase
Incidental Oral Intermediate-Term (1 - 6 Months) Residential Only	NOAEL= **1** **MOE** = TBD	1	**Subchronic Neurotoxicity - rat** LOAEL = 10 mg/kg/day based on increased incidence of FOB changes; decrease in RBC, whole blood, plasma and brain cholinesterase.
Non-Dietary Risk Assessments			
Dermal Short-Term (1 - 30 days)	Dermal NOAEL= **20**		**4-week dermal toxicity - rat** systemic LOAEL = 50 mg/kg/day based on statistically significant decreases in RBC cholinesterase in males and females and brain cholinesterase in males.
Residential	**MOE** = TBD	1	

Exposure Scenario	Dose (mg/kg/day) UF /MOE	Hazard Based Special FQPA Safety Factor	Endpoint for Risk Assessment
Occupational	100	1	
Dermal Intermediate-Term (1 - 6 Months)	Dermal NOAEL= **20**		**4-week dermal toxicity - rat** systemic LOAEL = 50 mg/kg/day based on statistically significant decreases in RBC cholinesterase in males and females and brain cholinesterase in males.
Residential	**MOE** = TBD	1	
Occupational	100	1	
Dermal Long-Term[a] (> 6 Months)	Oral NOAEL= **3.1**		**Chronic toxicity - dog** LOAEL = 3.1 mg/kg/day based on plasma and brain cholinesterase inhibition in females.
Residential	**MOE** = TBD	1	
Occupational	300	1	
Inhalation Short-Term [b] (1 - 30 days)	Oral NOAEL= **1**		**Developmental Neurotoxicity - rat** LOAEL = 10 mg/kg/day based on an increased incidence of FOB changes and statistically significant decreases in RBC, whole blood, plasma and brain cholinesterase
Residential	**MOE** = TBD	1	
Occupational	100	1	
Inhalation Intermediate-Term[b] (1 - 6 Months)	Oral NOAEL= **1**		**Subchronic Neurotoxicity - rat** LOAEL = 10 mg/kg/day based on increased incidence of FOB changes; decrease in RBC, whole blood, plasma and brain cholinesterase.
Residential	**MOE** = TBD	1	
Occupational	100	1	
Inhalation Long-Term[b] (>6 Months)	Oral NOAEL= **3.1**		**Chronic toxicity - dog** LOAEL = 3.1 mg/kg/day based on plasma and brain cholinesterase inhibition in females.
Residential	**MOE** = TBD	1	
Occupational	300	1	
Cancer	**Classification:** **Q1* =** 8.75×10^{-4}		

a Since an oral NOAEL/LOAEL was selected, a dermal absorption factor of 12.7% should be used in route-to-route extrapolation.

b Since an oral NOAEL was selected, an inhalation factor of 100% should be used in route-to-route extrapolation.

TBD = To Be Determined. Target MOEs for residential exposures will be determined by the FQPA Safety Factor Committee.